Supporting Information for:

Preparation of Translationally Competent tRNA by Direct Chemical Acylation

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Acylation of tRNA. Acylation reaction conditions as follows: 10-30 µg tRNA (~4-12 µM), 80 mM HEPES pH 7.5, 15-80 mM amino acid ethyl phosphate, 15-80 mM $La(OTf)_3$ (1 equivalent to aaEP) in a final volume of 100 µL. Addition of La^{3+} starts the reaction and generates insoluble complexes. The reaction is stirred vigorously at room temperature for 30 minutes. The reaction is quenched by addition of 50 µL of 300 mM diethylene triamine pentaacetic acid (DTPA) and 1.25 M NH_4OAc pH 7.5 (final concentrations 100 mM DTPA and 417 mM NH_4OAc). Excess amino acid is extracted with 1 volume of 25:24:1 phenol:chloroform:isoamyl alcohol pH 5.2. The organic layer was then extracted with 0.5 volume of DTPA/NH_4OAc quench solution. The combined aqueous solutions were then washed with 1 volume of 24:1 chloroform:isoamyl alcohol. tRNA was then precipitated by addition of 3 volumes of ethanol and storage at -20 °C overnight. tRNA was pelleted by centrifugation and dissolved in 1 mM NaOAc pH 4.5, followed by removal of co-precipitated DTPA-La using a CHROMA SPIN DEPC-water gel filtration column (Clontech, Mountain View, CA). The resulting tRNA solution was used directly for MALDI-MS analysis or protein expression.

Protein expression in Xenopus oocytes. Mouse muscle α1, β1, δ, and γ; and rat neuronal α4 and β2 nAChR cDNA in the pAMV vector was linearized with the restriction enzyme Not *I*. mRNA was prepared by *in vitro* transcription using the mMessage Machine T7 kit (Ambion, Austin, TX). Unnatural mutations were introduced by the standard Stratagene QuickChange protocol using a TAG or TGA mutation at the site of interest. The α4 subunit contained a known mutation in M2 transmembrane helix (L9'A). Stage V-VI *Xenopus laevis* oocytes were injected with mRNA in a 10:2:1:1 for $\alpha 1_2\beta 1_2\delta\gamma$ or 1:20 for $\alpha 4_2\beta 2_3$. Stoichiometry of α4β2 confirmed by monitoring I-V relationships of acetylcholine-induced currents as described previously.[1] Each cell was injected with 50 nL of a mixture of mRNA (10-19 ng, typically ~13 ng): tRNA (3-42 ng, typically ~27 ng). Uncharged full length tRNA was injected as a negative control.

Electrophysiology. Electrophysiology experiments were performed 24-48 hours after injection using the OpusXpress 6000A instrument (Axon Instruments, Union City, CA) in two-electrode voltage clamp mode at a holding potential of -60 mV. The running buffer was Ca^{2+}-free ND96 solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$ and 5 mM HEPES, pH 7.5). Oocytes were superfused with running buffer at 1 mL/min for 30s before application of acetylcholine for 15 s followed by a 116 s wash with the running buffer. Data were sampled at 125 Hz and filtered at 50 Hz. Acetylcholine chloride was purchased from Sigma-Aldrich/RBI (St. Louis, MO). Does-response data were obtained for ≥ 10 acetylcholine concentrations on ≥ 15 cells. All EC_{50} and Hill coefficient values were obtained by fitting does-response relations to the Hill equation ($I_{norm} = 1 / [1 + (EC_{50}/[ACh])^n]$) and are reported as means ± standard error of the fit. A detailed error analysis of nonsense suppression experiments reveals data are reproducible to ±50% in EC_{50}.[2]

Synthesis

Tetraethylammonium NVOC-leucine ethyl phosphate (**1**). A round bottom flask was charged with α-NVOC-leucine (170 mg, 0.46 mmol) and *N,N'*-dicyclohexylcarbodiimide (94 mg, 0.46 mmol) in 10 mL of dry CH_2Cl_2. Reaction allowed to stir for 10 minutes at room temperature. A separately prepared solution of bis(tetraethylammonium) ethyl phosphate (175 mg, 0.46 mmol) in 10 mL dry CH_2Cl_2 was added via syringe. Reaction allowed to stir for 4 hours at room temperature. The reaction mixture was concentrated and resuspended in 5 mL of CH_2Cl_2. Upon filtration, the organic solution was extracted three times with 5 mL of water. Saturated NaCl solution was added dropwise to aid in emulsion breaking. The combined aqueous extracts were washed with 5 mL $CHCl_3$ followed by lyophilization. The resulting solid was extracted with CH_2Cl_2 and filtered. Concentration of the CH_2Cl_2 solution gave a yellow foam 182 mg (66 %). Analytical sample obtained by reverse phase HPLC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.76 (d, *J* = 8.9 Hz, 1H), 7.68 (s, 1H), 7.17 (s, 1H), 5.34 (s, 2H), 4.03 – 3.91 (m, 1H), 3.90 (s, 3H), 3.84 (s, 3H), 3.70 (dt, *J* = 14.3, 7.2 Hz, 2H), 3.17 (q, *J* = 7.3 Hz, 8H), 1.72 – 1.58 (m, 1H), 1.54 – 1.45 (m, 2H), 1.18 – 1.08 (m, 12H), 1.03 (t, *J* = 7.1 Hz, 3H), 0.85 (dd, *J* = 12.9, 6.5 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.69, 169.62, 155.57, 154.01, 147.91, 139.09, 128.76, 109.53, 107.99, 63.35, 61.81, 61.76, 56.80, 56.43, 53.62, 53.58, 52.59, 41.35, 24.82, 23.08, 21.61, 16.64, 16.58, 7.78. HRMS ES (–) *m/z* for $C_{18}H_{26}N_2O_{11}P$, found 477.1259, calculated 477.1274.

Tetraethylammonium N-NVOC-tyrosine ethyl phosphate (**2**). A round bottom flask was charged with α-NVOC-tyrosine (150 mg, 0.36 mmol) and *N,N'*-dicyclohexylcarbodiimide (147 mg, 0.71 mmol) in 1.5 mL

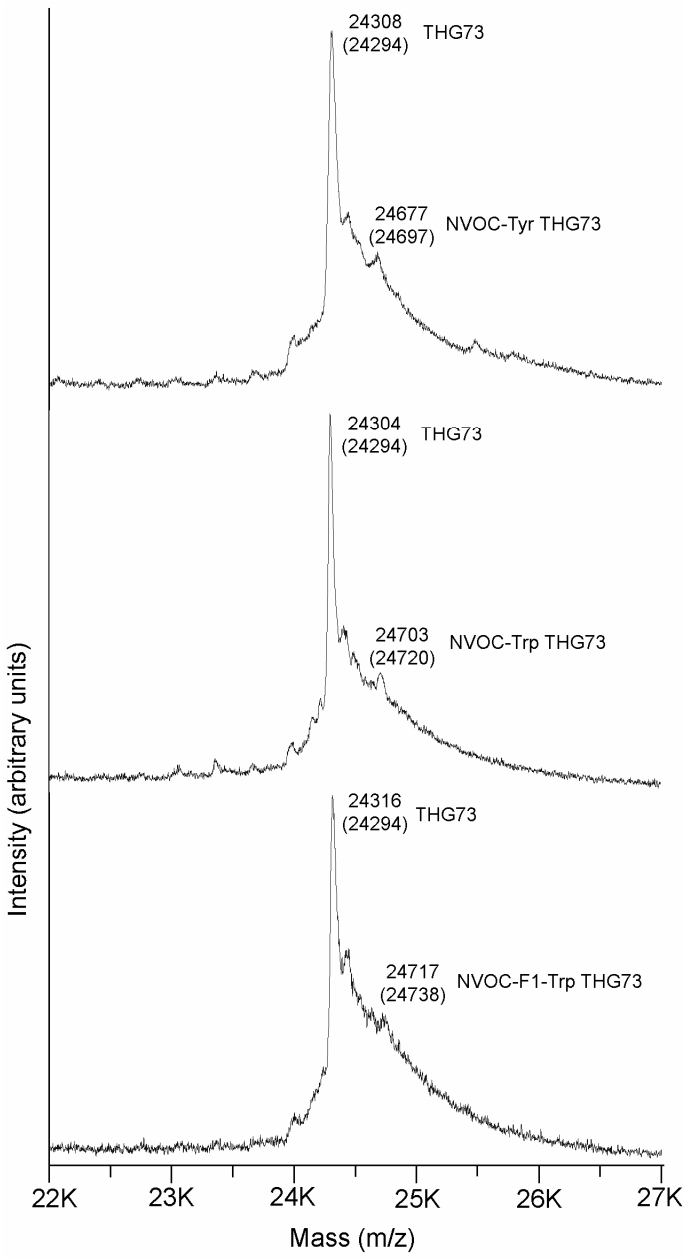
of dry THF and 1 mL of dry CH_2Cl_2. Reaction allowed to stir for 4 minutes at room temperature. A separately prepared solution of bis(tetraethylammonium) ethyl phosphate (91 mg, 0.24 mmol) in 4 mL dry CH_2Cl_2 was added via syringe. Reaction allowed to stir for 19 hours at room temperature. The reaction mixture was filtered, and extracted three times with 5 mL of water. Saturated NaCl solution was added dropwise to aid in emulsion breaking. The combined aqueous extracts were lyophilized. The resulting solid was extracted with acetonitrile and filtered. Concentration of the acetonitrile solution gave a yellow oil 92 mg (63 %). Analytical sample obtained by reverse phase HPLC. ^{1}H NMR (300 MHz, CD_3CN) δ 8.93 (br, 1H), 7.69 (s, 1H), 7.07 (d, *J* = 8.6 Hz, 3H), 6.78 (d, *J* = 8.5 Hz, 2H), 6.13 (d, *J* = 8.5 Hz, 1H), 5.39 (s, 2H), 4.44 – 4.32 (m, 1H), 4.05 – 3.79 (m, *J* = 12.5, 5.1 Hz, 8H), 3.18 (q, *J* = 7.3 Hz, 8H), 3.14 – 3.05 (m, 1H), 2.99 – 2.84 (m, 1H), 1.28 – 1.15 (m, 15H). ^{13}C NMR (126 MHz, CD_3CN) δ 168.88, 168.80, 157.53, 155.99, 154.27, 147.96, 139.02, 130.28, 128.93, 127.23, 117.50, 115.60, 109.63, 108.09, 62.96, 61.45, 61.40, 57.13, 56.62, 56.00, 52.24, 52.22, 52.19, 35.89, 16.18, 16.12, 6.93. ^{31}P NMR (121 MHz, CD_3CN) δ -8.52. HRMS ES (–) *m/z* for $C_{21}H_{24}N_2O_{12}P$, found 527.1062, calculated 527.1067.

Tetraethylammonium NVOC-tryptophan ethyl phosphate (**3**). A round bottom flask was charged with α-NVOC-tryptophan (107 mg, 0.24 mmol) and *N,N'*-dicyclohexylcarbodiimide (50 mg, 0.24 mmol) in 6 mL of dry THF. Reaction allowed to stir for 10 minutes. A separately prepared solution of bis(tetraethylammonium) ethyl phosphate (93 mg, 0.25 mmol) in 5 mL CH_2Cl_2 was then added via syringe. Reaction allowed to stir overnight at room temperature. The reaction mixture was concentrated on a rotary evaporator and resuspended in 2.5 mL of CH_2Cl_2. Filtration of the suspension gave an organic solution that was extracted three times with 2 mL of water. Combined aqueous extracts were washed with 2 mL of $CHCl_3$ followed by lyophilization to give a yellow foam, 87.9 mg (55%). Analytical sample obtained by reverse phase HPLC. ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.41 (s, 1H), 7.67 (s, 1H), 7.62 – 7.42 (m, 3H), 7.12 – 6.88 (m, 3H), 5.58 (d, *J* = 8.2 Hz, 1H), 5.47 (s, 2H), 4.80 – 4.60 (m, 1H), 4.18 – 4.01 (m, 2H), 3.96 (s, 3H), 3.81 (s, 3H), 3.42 (d, *J* = 4.6 Hz, 2H), 2.93 (q, *J* = 7.3 Hz, 8H), 1.28 (t, *J* = 7.0 Hz, 3H), 1.01 (t, *J* = 7.1 Hz, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.76, 168.63, 155.30, 153.81, 148.02, 139.33, 136.58, 128.41, 128.36, 125.17, 120.96, 118.74, 117.72, 112.43, 110.09, 108.07, 107.85, 77.45, 77.02, 76.60, 63.40, 62.13, 62.04, 56.60, 56.39, 52.25, 27.15, 16.68, 16.58, 7.25. ^{31}P NMR (121 MHz, $CDCl_3$) δ -7.76. HRMS ES (–) *m/z* for $C_{23}H_{25}N_3O_{11}P$, found 550.1229, calculated 550.1227.

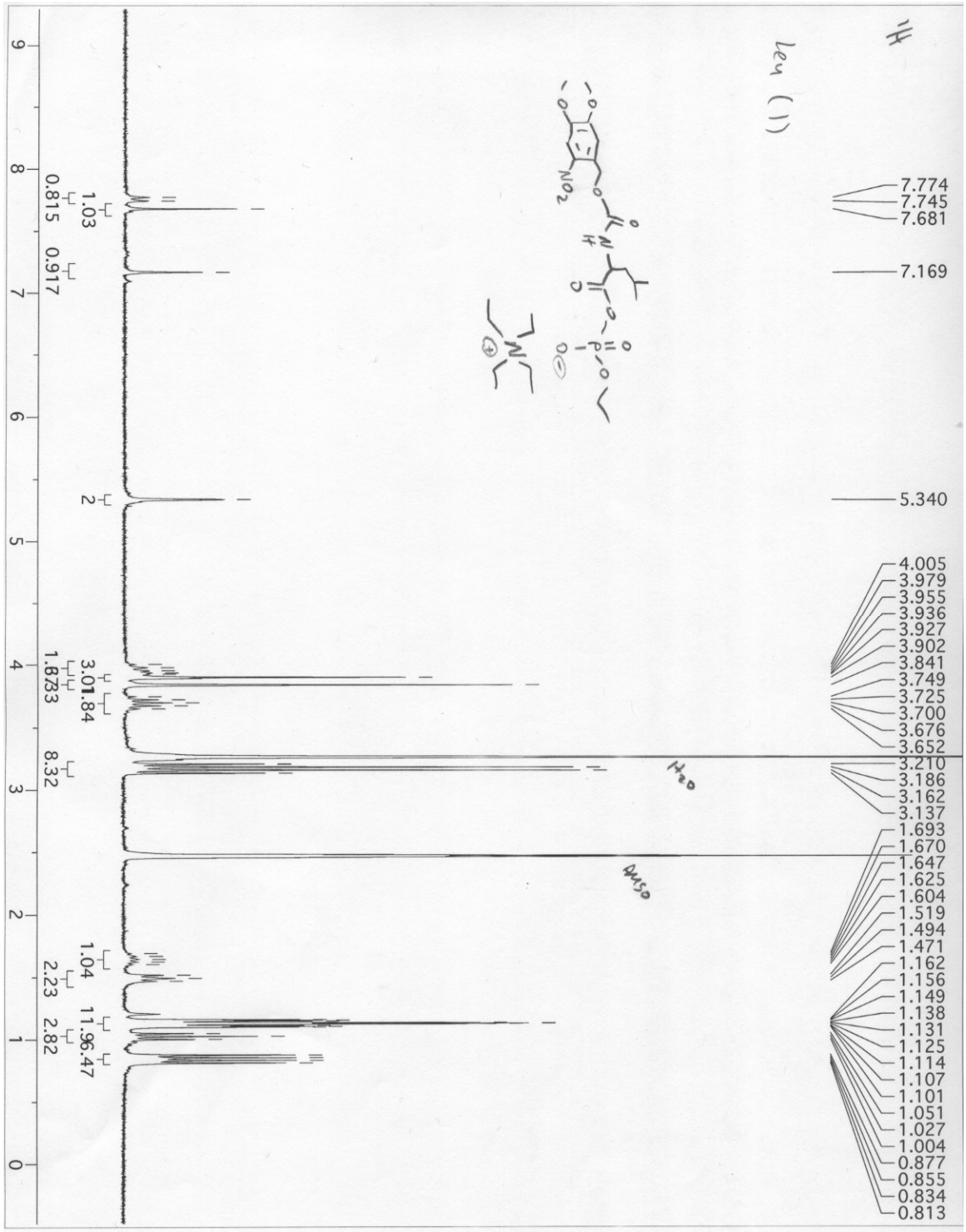
Tetraethylammonium NVOC-5-fluorotryptophan ethyl phosphate (**4**). A round bottom flask was charged with α-NVOC-5-fluorotryptophan (82 mg, 0.18 mmol) and *N,N'*-dicyclohexylcarbodiimide (42 mg, 0.20 mmol) in 5 mL of dry THF. Reaction allowed to stir for 10 minutes at room temperature. A separately prepared solution of bis(tetraethylammonium) ethyl phosphate (72 mg, 0.19 mmol) in 5 mL dry CH_2Cl_2 was added via syringe. Reaction allowed to stir for 24 hours at room temperature. The reaction mixture was concentrated and resuspended in 3 mL of CH_2Cl_2. Upon filtration, the organic solution was extracted three times with 4 mL of water. Saturated NaCl solution was added dropwise to aid in emulsion breaking. The combined aqueous extracts were lyophilized. The resulting solid was extracted with CH_2Cl_2 and filtered. Concentration of the CH_2Cl_2 solution gave a yellow foam 55 mg (44 %). Analytical sample obtained by reverse phase HPLC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.71 (s, 1H), 7.66 (s, 1H), 7.58 (d, *J* = 2.1 Hz, 1H), 7.53 (dd, *J* = 8.7, 4.6 Hz, 1H), 7.09 (dd, *J* = 9.9, 2.3 Hz, 1H), 6.95 (s, 1H), 6.79 (td, *J* = 9.1, 2.4 Hz, 1H), 5.59 (d, *J* = 8.2 Hz, 1H), 5.45 (d, *J* = 4.0 Hz, 2H), 4.70 – 4.62 (m, 1H), 4.14 – 4.04 (m, 2H), 3.93 (s, 3H), 3.86 (s, 3H), 3.35 (ddd, *J* = 20.0, 14.9, 4.6 Hz, 2H), 2.98 (q, *J* = 7.3 Hz, 8H), 1.28 (t, *J* = 7.1 Hz, 3H), 1.03 (t, *J* = 7.2 Hz, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.75, 168.68, 158.52, 156.67, 155.32, 153.79, 148.02, 139.42, 133.12, 128.51, 128.44, 128.12, 127.17, 113.18, 113.11, 110.08, 109.25, 109.04, 108.02, 107.84, 107.81, 102.35, 102.16, 63.52, 62.19, 62.14, 56.53, 56.37, 52.23, 27.08, 16.62, 16.56, 7.23. ^{31}P NMR (162 MHz, $CDCl_3$) δ -7.83. HRMS ES (–) *m/z* for $C_{23}H_{24}N_3O_{11}FP$, found 568.1110, calculated 568.1133.

References:

(1) Xiu, X. A.; Puskar, N. L.; Shanata, J. A. P.; Lester, H. A.; Dougherty, D. A. *Nature* **2009**, *458*, 534.
(2) Torrice, M. M.; Bower, K. S.; Lester, H. A.; Dougherty, D. A. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 11919.



Supplemental Figure 1 MALDI mass spectra of THG73 tRNA after (below) exposure to La^{3+} mediated acylation conditions using tyrosine derivative **2** (top), tryptophan derivative **3** (middle), and fluro-tryptophan derivative **4** (bottom). Observed masses shown, theoretical masses in parentheses. Based on calibration data, we estimate the yields at 15%, 10%, and 5% for **2**, **3**, and **4**, respectively.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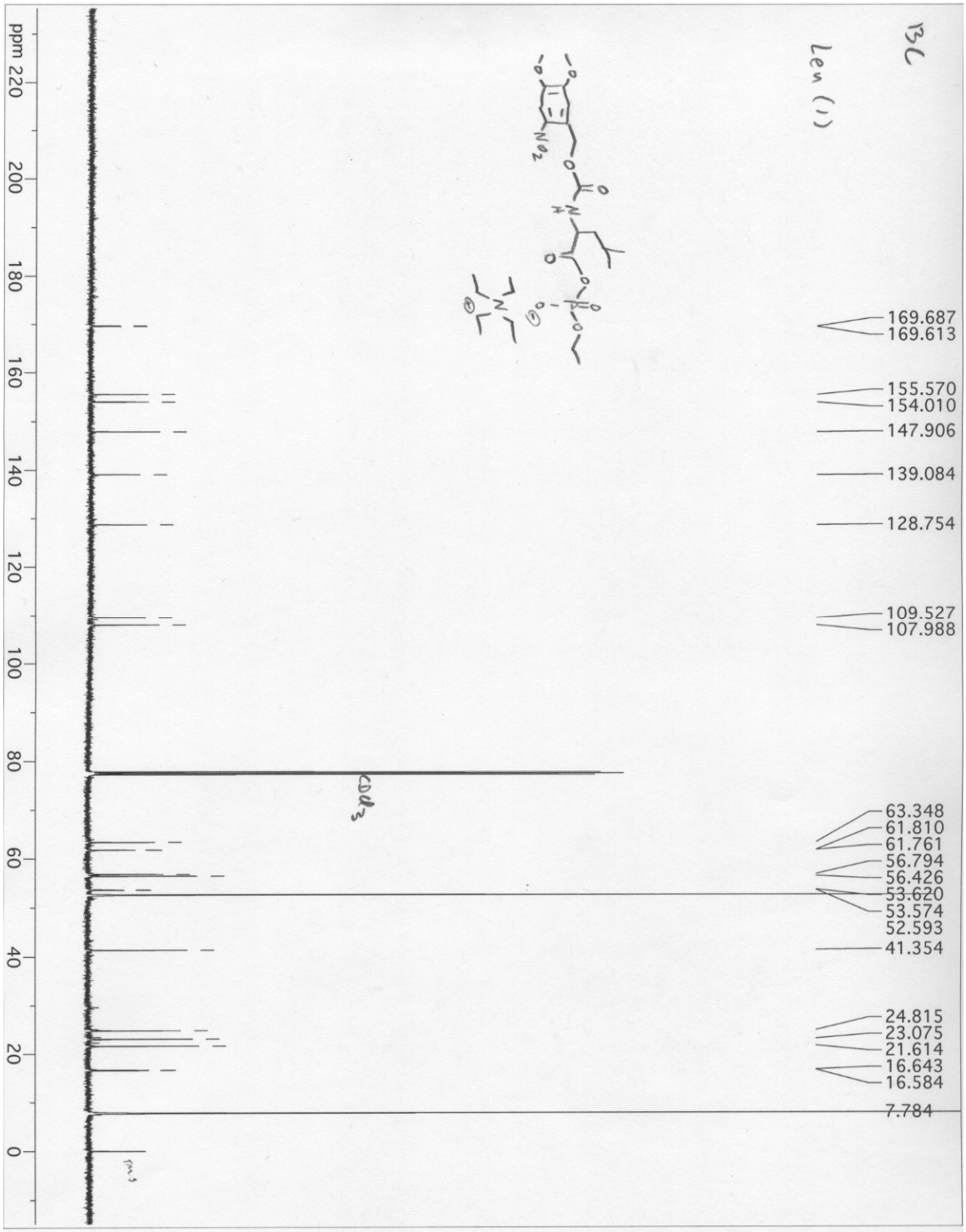
Leu (1)
7.774
7.745
7.681
7.169
5.340
4.005
3.979
3.955
3.936
3.927
3.902
3.841
3.749
3.725
3.700
3.676
3.652
3.210
3.186
3.162
3.137
1.693
1.670
1.647
1.625
1.604
1.519
1.494
1.471
1.162
1.156
1.149
1.138
1.131
1.125
1.114
1.107
1.101
1.051
1.027
1.004
0.877
0.855
0.834
0.813
H2O
DMSO
1.03
0.815
0.917
2
3.01
1.84
1.67
33
8.32
1.04
11.96
47
2.23
2.82


13C
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155.570
154.010
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139.084
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109.527
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61.810
61.761
56.794
56.426
53.620
53.574
52.593
41.354
24.815
23.075
21.614
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CDCl3
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180
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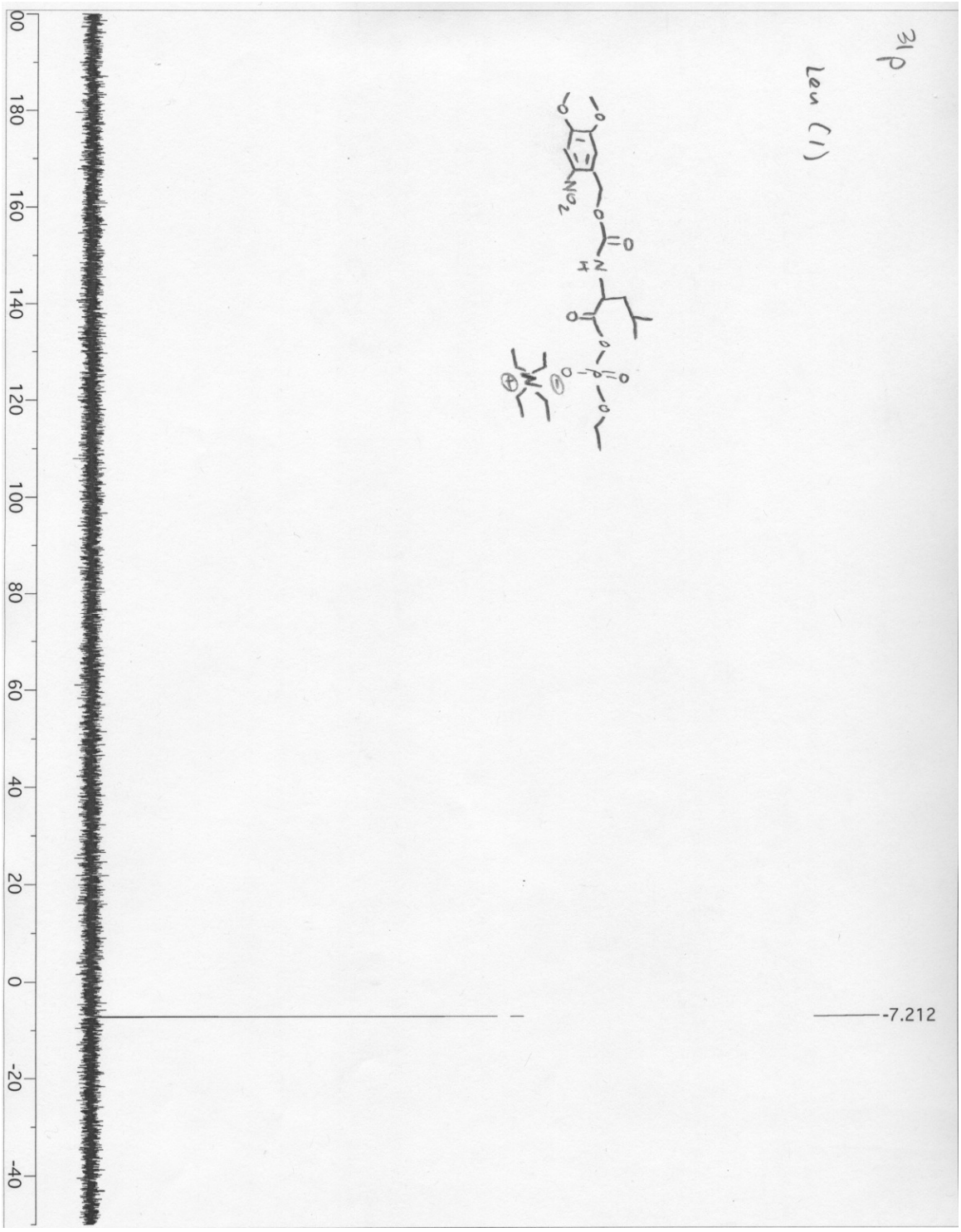
140
120
100
80
60
40
20
0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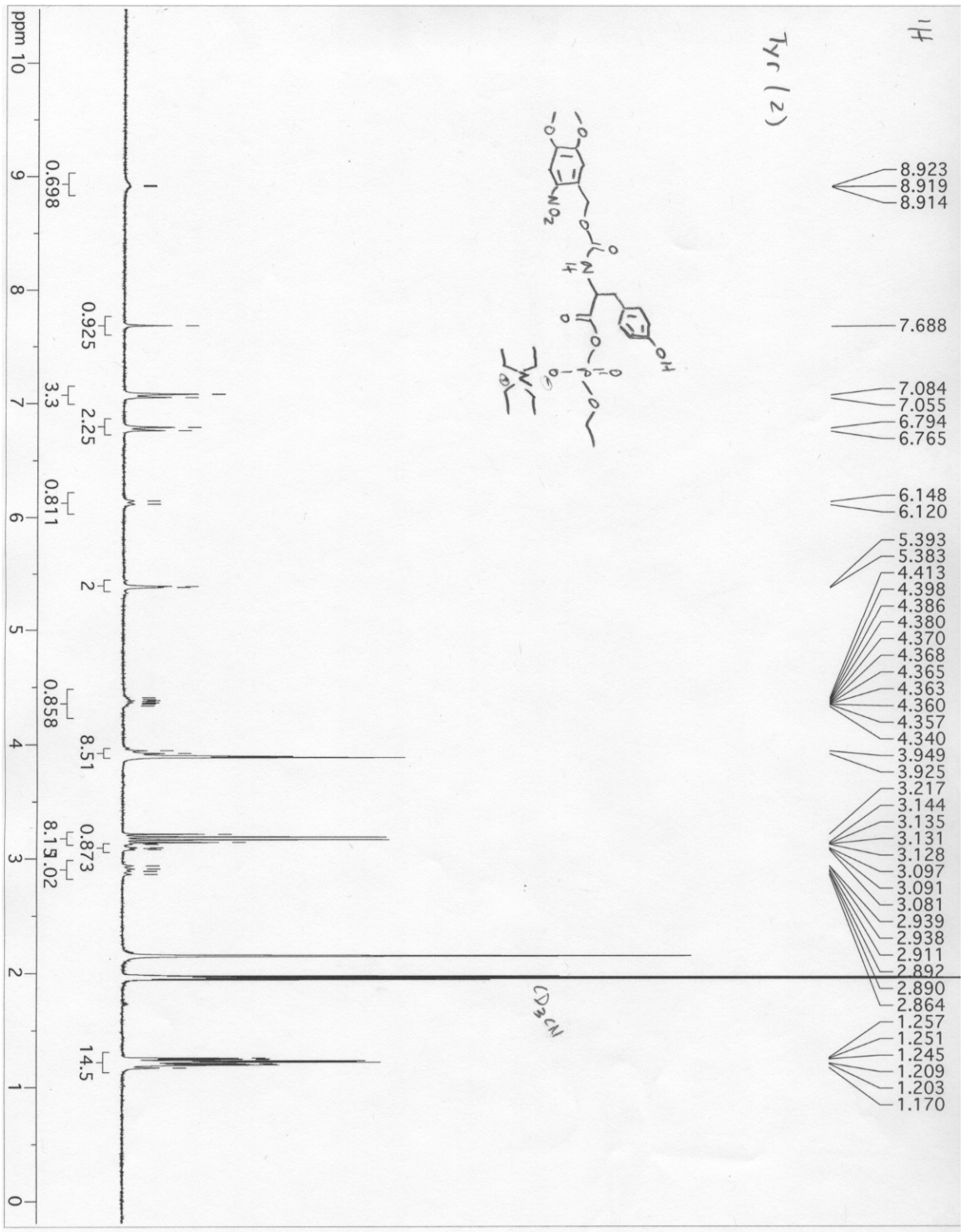
100
80
60
40
20
0
-20
-40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Tyr (2)
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8.919
8.914
7.688
7.084
7.055
6.794
6.765
6.148
6.120
5.393
5.383
4.413
4.398
4.386
4.380
4.370
4.368
4.365
4.363
4.360
4.357
4.340
3.949
3.925
3.217
3.144
3.135
3.131
3.128
3.097
3.091
3.081
2.939
2.938
2.911
2.892
2.890
2.864
1.257
1.251
1.245
1.209
1.203
1.170
(CD3CN)
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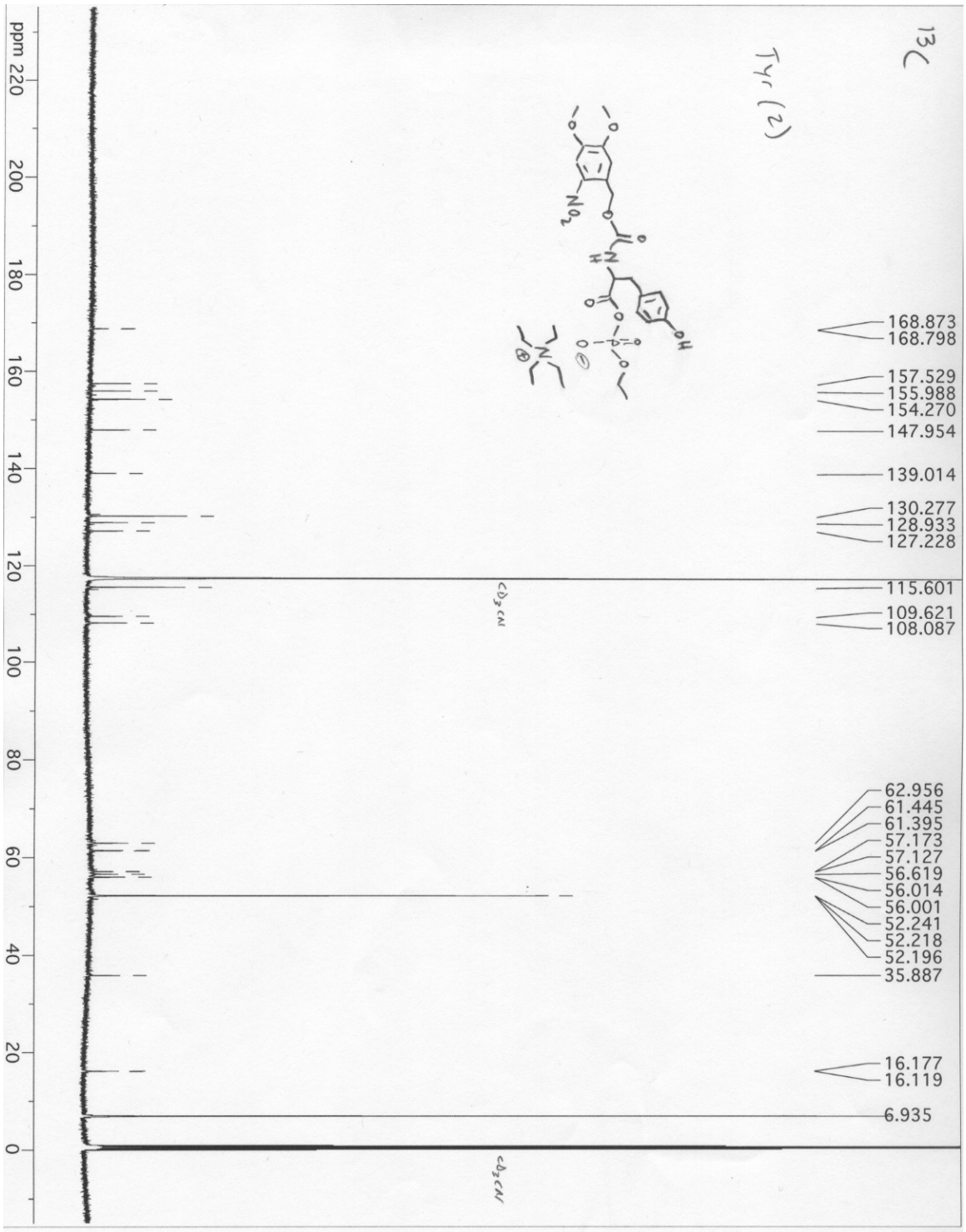
0.811
2
0.858
8.51
0.873
8.13
3.02
14.5


13C
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157.529
155.988
154.270
147.954
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130.277
128.933
127.228
115.601
109.621
108.087
62.956
61.445
61.395
57.173
57.127
56.619
56.014
56.001
52.241
52.218
52.196
35.887
16.177
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CD3CN
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40
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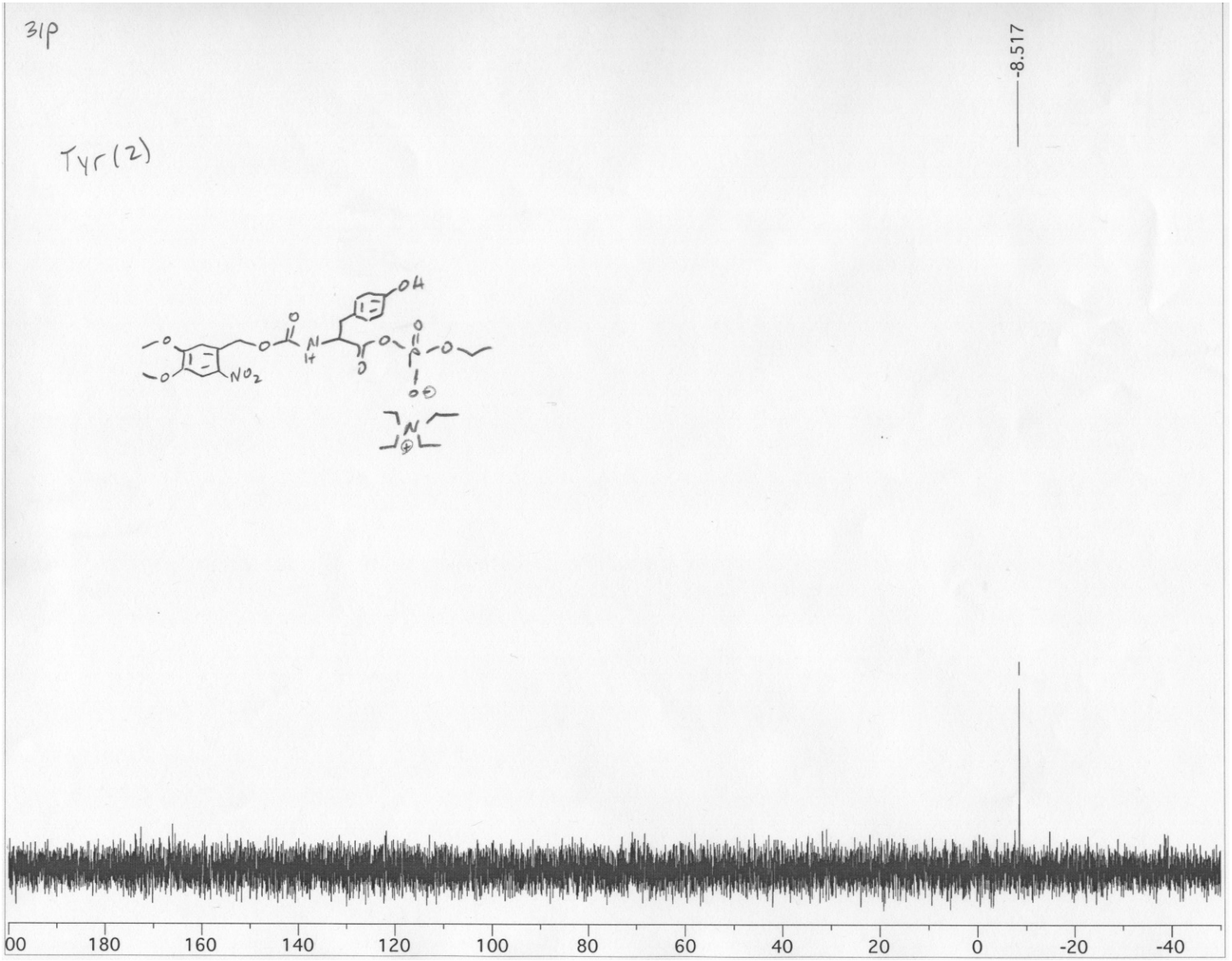
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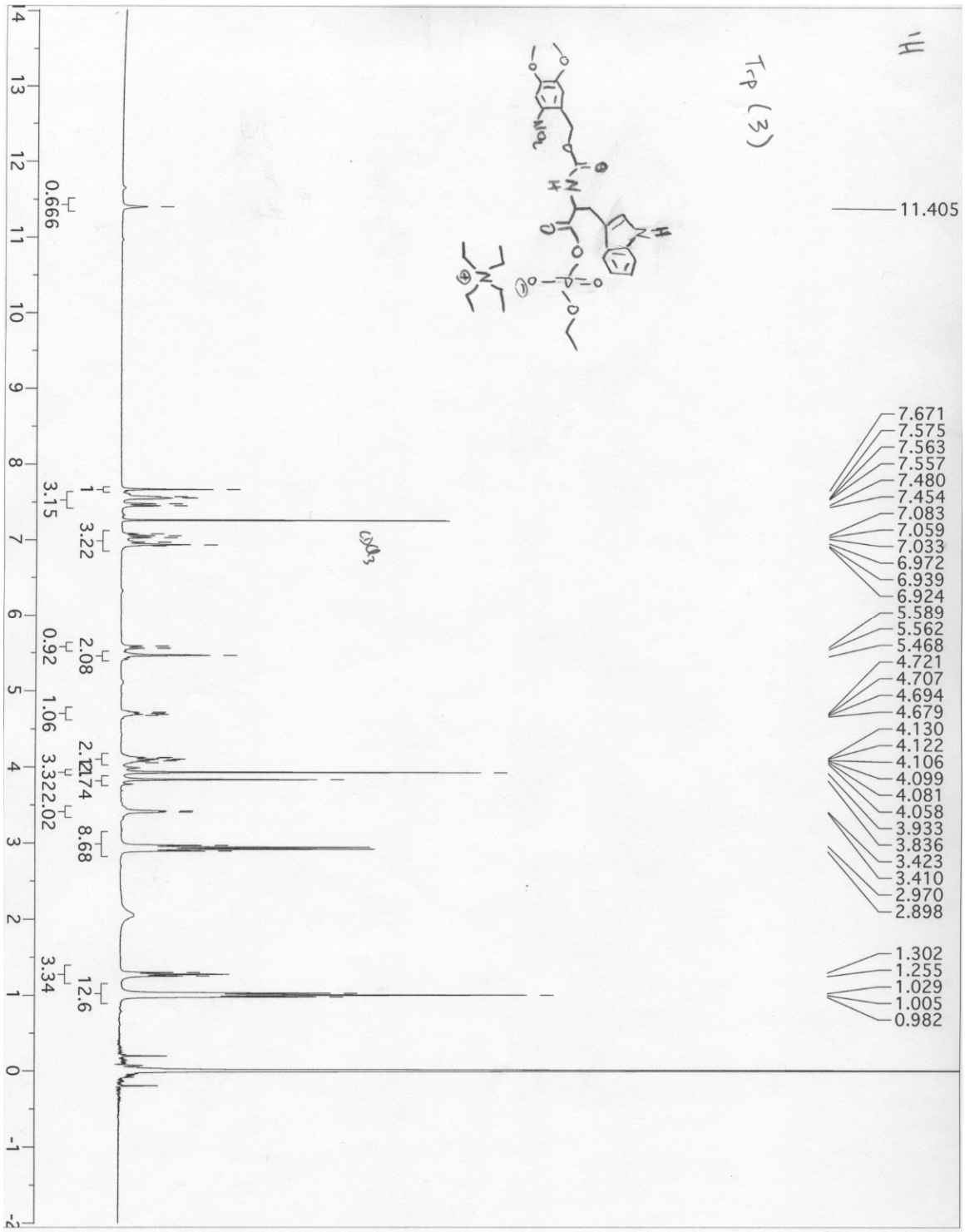
-20
-40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Trp (3)
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6.939
6.924
5.589
5.562
5.468
4.721
4.707
4.694
4.679
4.130
4.122
4.106
4.099
4.081
4.058
3.933
3.836
3.423
3.410
2.970
2.898
1.302
1.255
1.029
1.005
0.982
0.666
3.15
1
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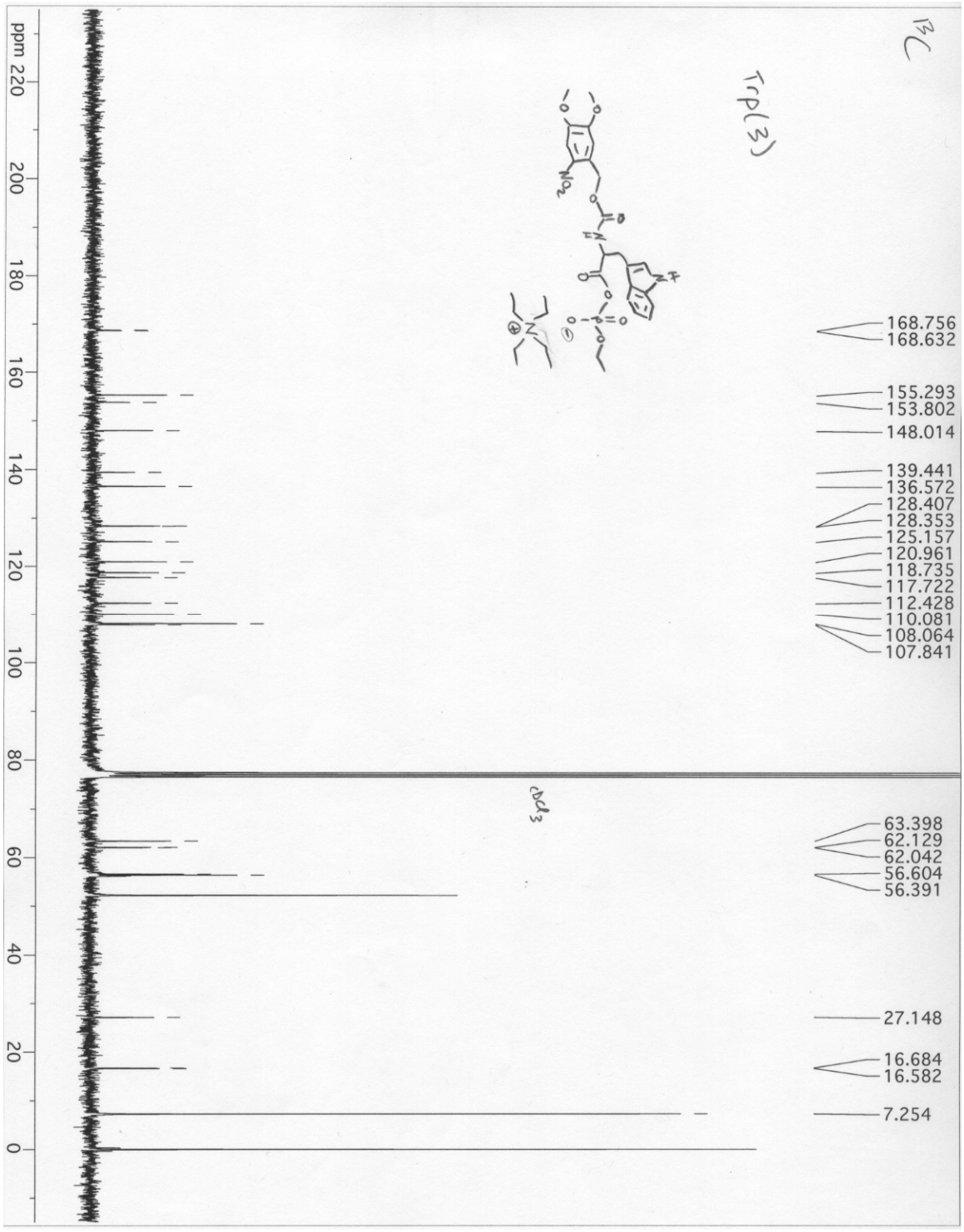
2.02
8.68
3.34
12.6
CDCl3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148.014
139.441
136.572
128.407
128.353
125.157
120.961
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117.722
112.428
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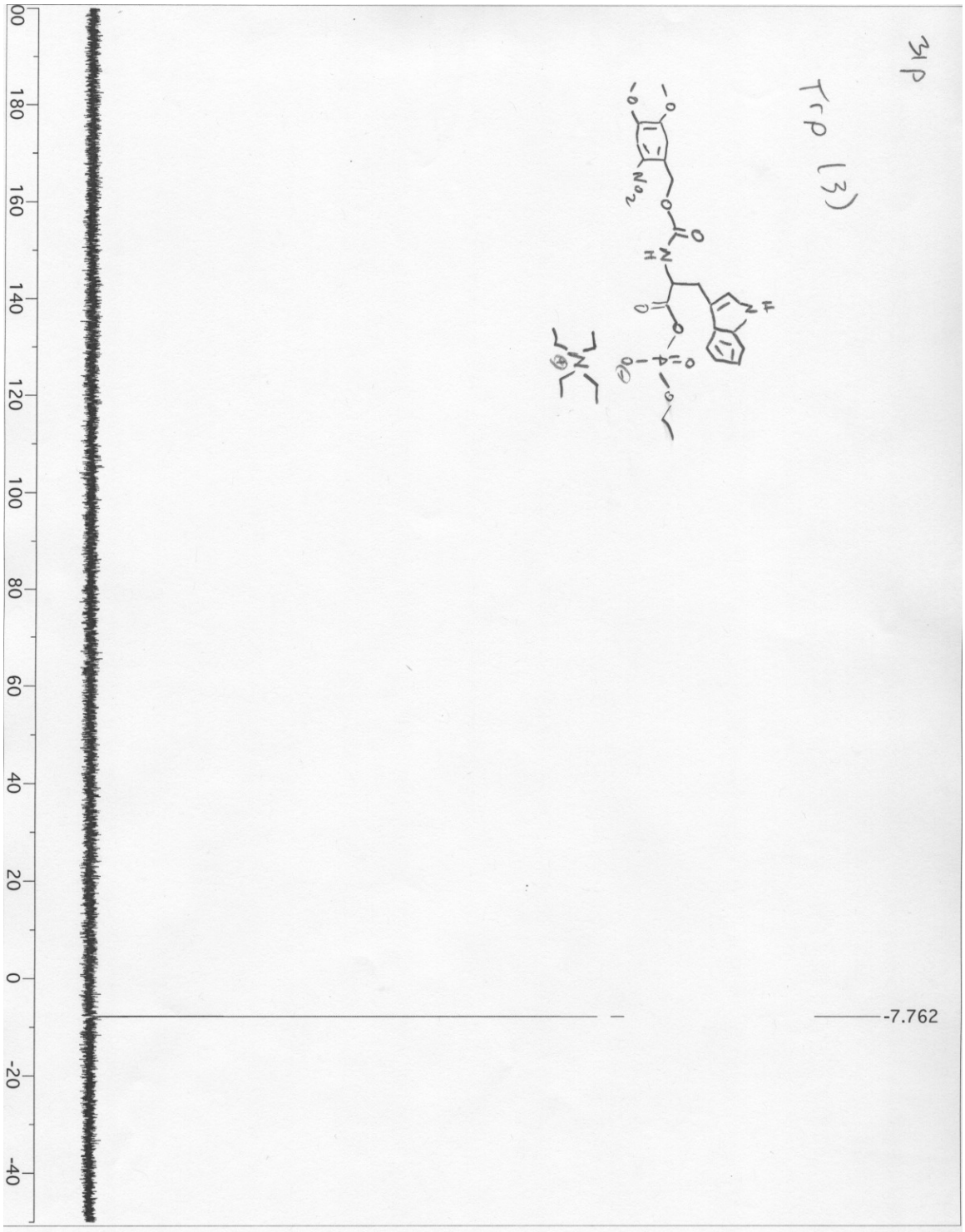
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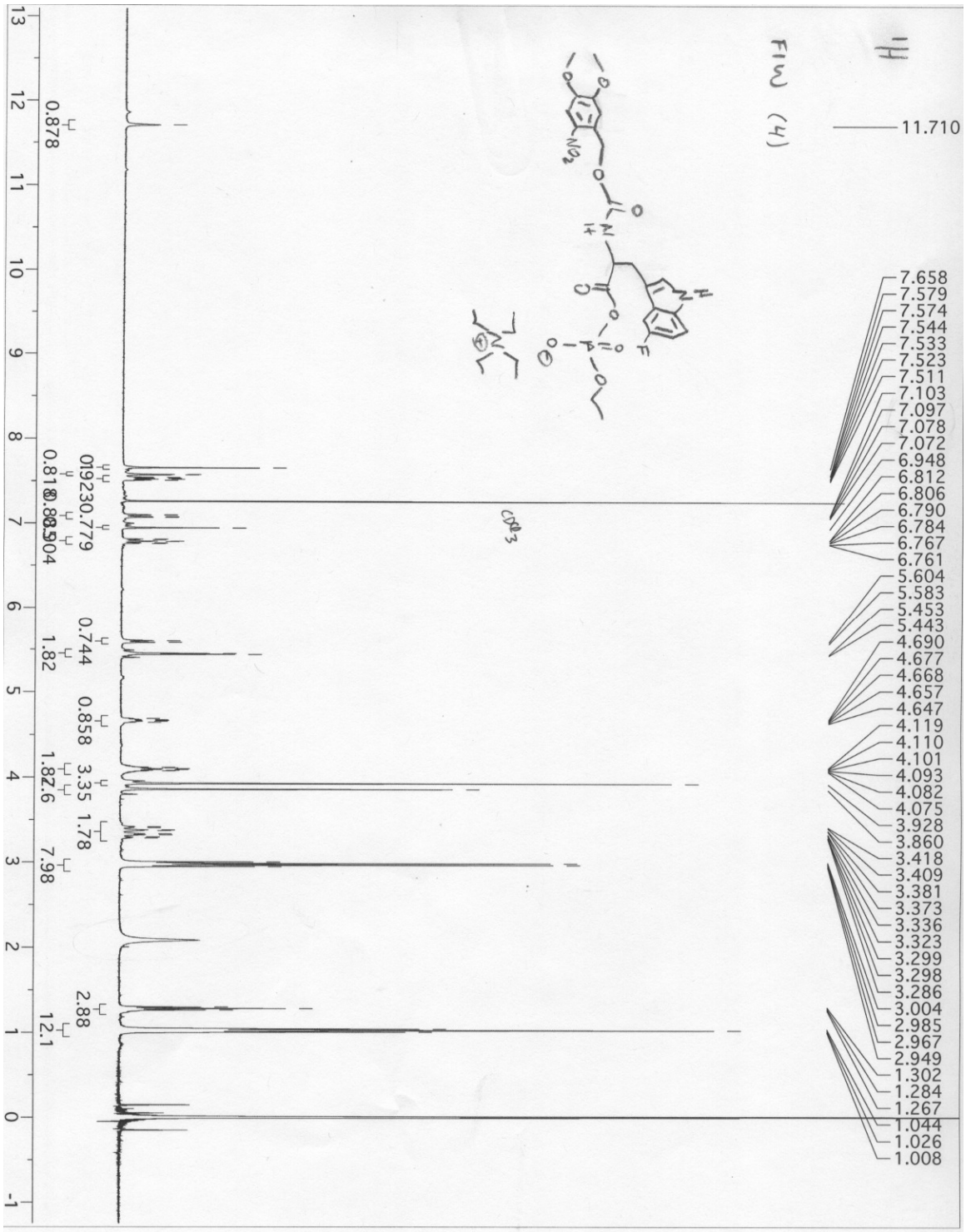
-40


1H
FIN (4)
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7.658
7.579
7.574
7.544
7.533
7.523
7.511
7.103
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7.072
6.948
6.812
6.806
6.790
6.784
6.767
6.761
5.604
5.583
5.453
5.443
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4.075
3.928
3.860
3.418
3.409
3.381
3.373
3.336
3.323
3.299
3.298
3.286
3.004
2.985
2.967
2.949
1.302
1.284
1.267
1.044
1.026
1.008
CDCl3
0.878
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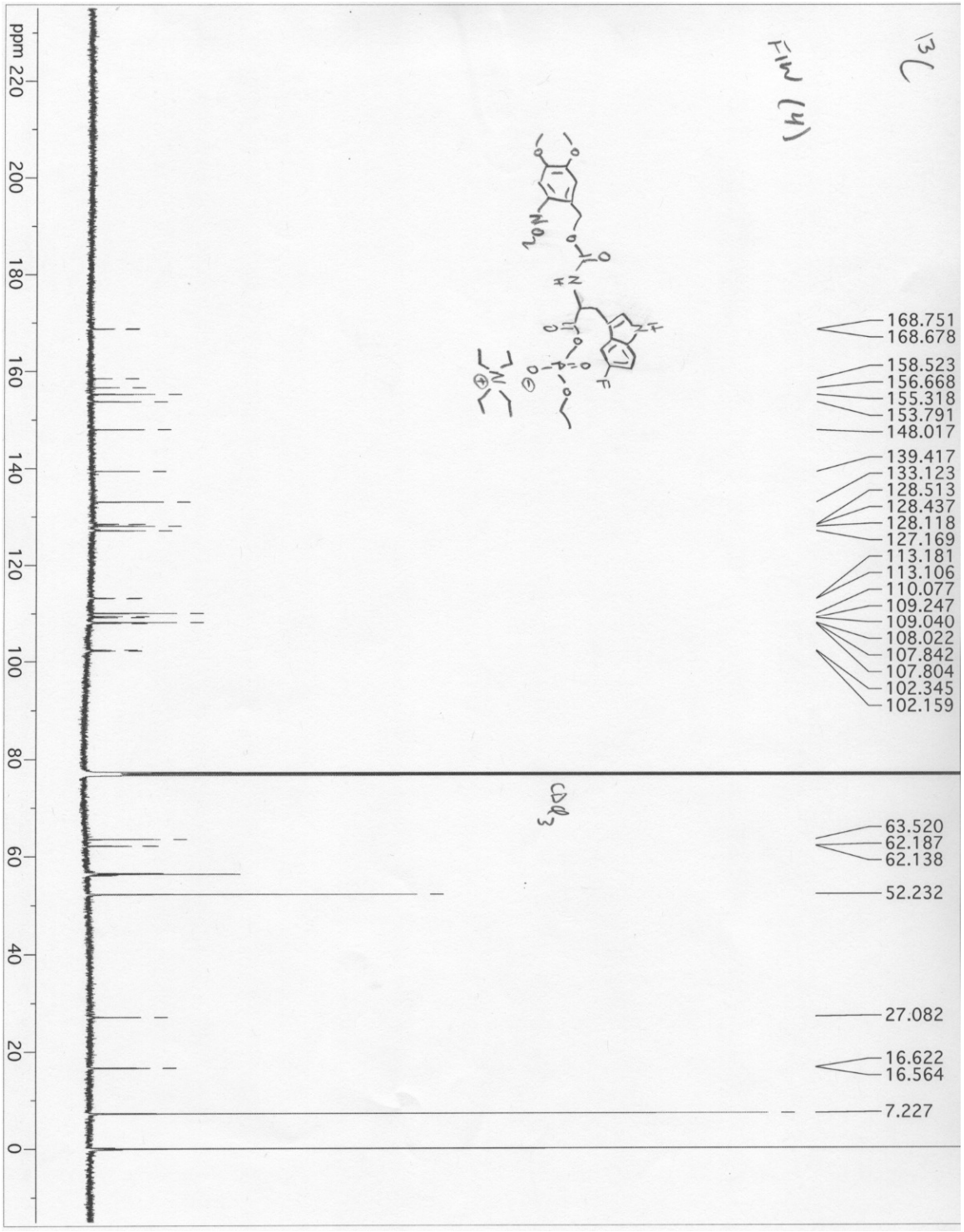
1.82
1.78
7.98
2.88
12.1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128.437
128.118
127.169
113.181
113.106
110.077
109.247
109.040
108.022
107.842
107.804
102.345
102.159
CDCl3
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62.187
62.138
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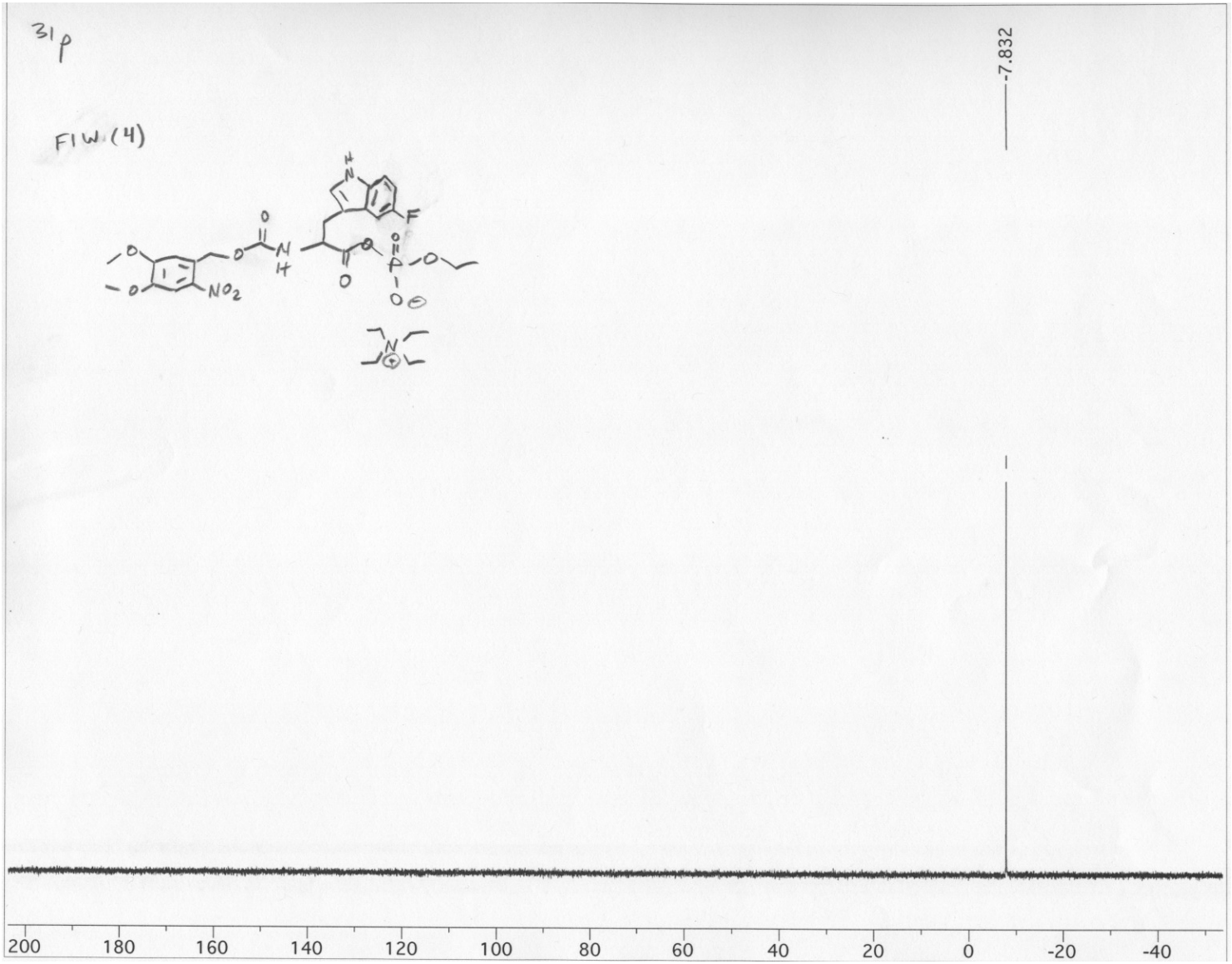
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FIW (4)
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140
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100
80
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20
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-20
-40